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Noninvasive Photoacoustic Computed Tomography of Mouse Brain Metabolism *In Vivo*

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ABSTRACT

To control the overall action of the body, brain consumes a large amount of energy in proportion to its volume. In humans and many other species, the brain gets most of its energy from oxygen-dependent metabolism of glucose. An abnormal metabolic rate of glucose and/or oxygen usually reflects a diseased status of brain, such as cancer or Alzheimer's disease. We have demonstrated the feasibility of imaging mouse brain metabolism using photoacoustic computed tomography (PACT), a fast, noninvasive and functional imaging modality with optical contrast and acoustic resolution. Brain responses to forepaw stimulations were imaged transdermally and transcranially. 2-NBDG, which diffuses well across the blood-brain-barrier, provided exogenous contrast for photoacoustic imaging of glucose response. Concurrently, hemoglobin provided endogenous contrast for photoacoustic imaging of hemodynamic response. Glucose and hemodynamic responses were quantitatively unmixed by using two-wavelength measurements. We found that glucose uptake and blood perfusion around the somatosensory region of the contralateral hemisphere were both increased by stimulations, indicating elevated neuron activity. The glucose response amplitude was about half that of the hemodynamic response. While the glucose response area was more homogenous and confined within the somatosensory region, the hemodynamic response area showed a clear vascular pattern and spread about twice as wide as that of the glucose response. The PACT of mouse brain metabolism was validated by high-resolution open-scalp OR-PAM and fluorescence imaging. Our results demonstrate that 2-NBDG-enhanced PACT is a promising tool for noninvasive studies of brain metabolism.

Keywords: Photoacoustic computed tomography, blood flow dynamics, glucose dynamics, functional brain imaging, glucose metabolism.

1. INTRODUCTION

In mammals, brain is the function center that coherently controls the overall action of the body. By performing varied computation-intensive tasks such as information processing, perception, motor control and learning, brain tissue consumes a large amount of energy in proportion to its volume. It is generally accepted that energy consumption is closely coupled with neuron activity: stronger neuron firing, more energy consumption [1]. This relationship has formed the basis for the functional brain imaging methods, including positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and near infrared spectroscopy (NIRS), to study brain physiology and pathology [2, 3].

For the first time, we have demonstrated that photoacoustic computed tomography (PACT) is able to image glucose uptake in the mouse brain, using a newly developed glucose analog 2-NBDG. To demonstrate the metabolic imaging capability of PACT, we studied *in vivo* forepaw stimulation responses. Our studies showed that PACT could spectrally

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separate 2-NBDG and blood using two-wavelength measurements, thus unmix the glucose and hemodynamic responses to the stimulations [4].

2. MATERIALS AND METHODS

2-NBDG is a newly developed fluorescent 2-deoxyglucose (2-DG) analog, shown in Figure 1a. Like the FDG (molecular weight: 181) used in PET studies, 2-NBDG is transported into cells via the same GLUT as glucose. The distribution of trapped 2-NBDG is a good reflection of glucose metabolism. The fluorescence imaging results show that tumor cells have a much faster 2-NBDG uptake rate, compared with the normal tissue cells, as shown in Figure 2. Because 2-NBDG is a relatively small molecule (molecular weight: 342), it crosses the blood-brain-barrier much more easily than other near-infrared fluorophore-labeled 2-DG analogs.

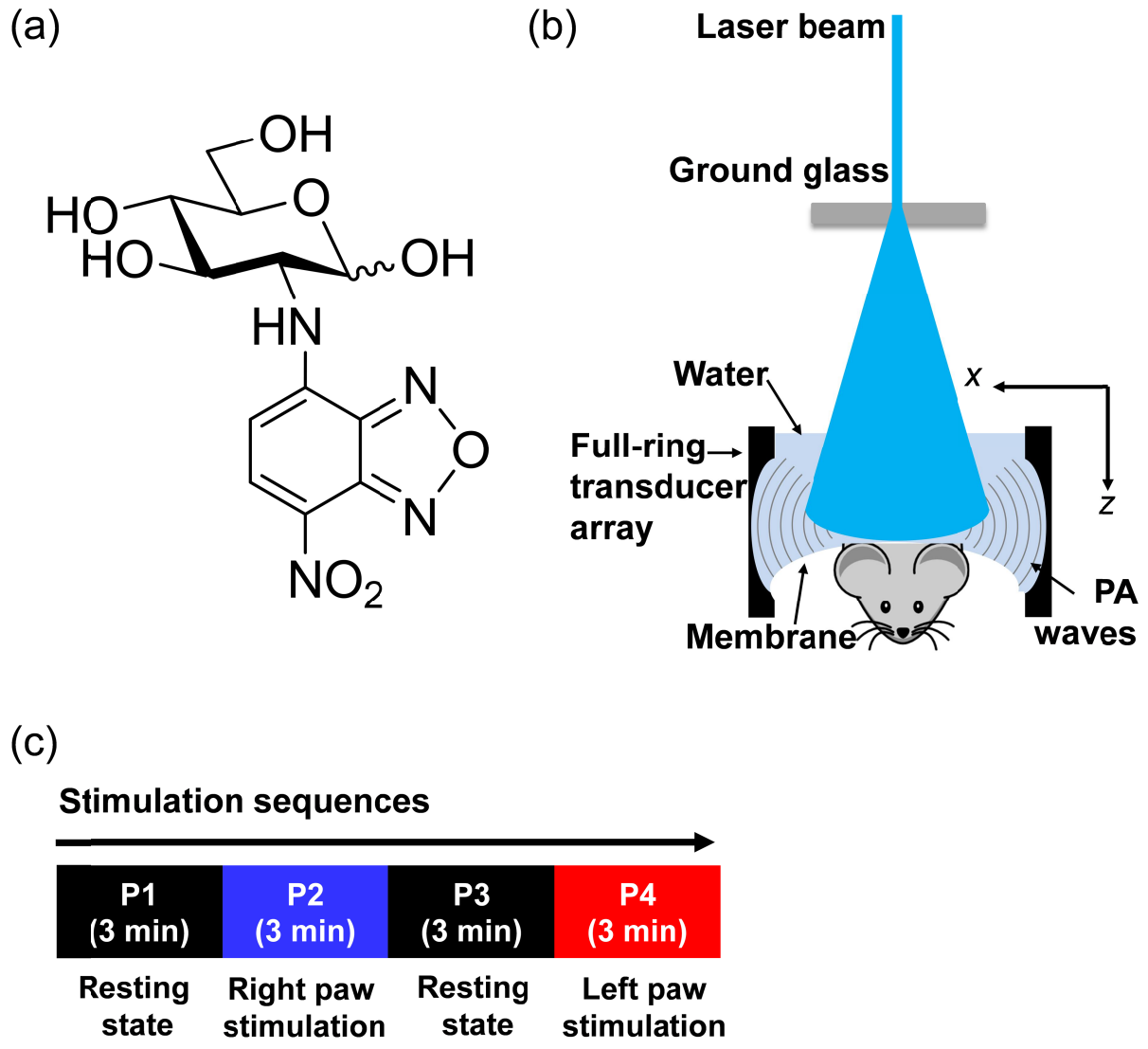

Figure 1.(a) 2-NBDG molecule. (b) Schematic of PACT system. (c) Electrical stimulation sequences.

Figure 1b is schematic of the PACT setup [5]. An OPO provides pulses with wavelengths tunable from 420 nm to 680 nm. The photoacoustic signals are detected by a 5 cm diameter full-ring ultrasonic transducer array with 512 elements. After a complete data acquisition, the raw data is reconstructed to form a photoacoustic image of the brain. The imaging speed is 1.6 sec per frame. Two wavelengths 478 nm and 570 nm were used to image the 2-NBDG and blood, respectively, based on their absorption spectra, shown in Figure 3a.

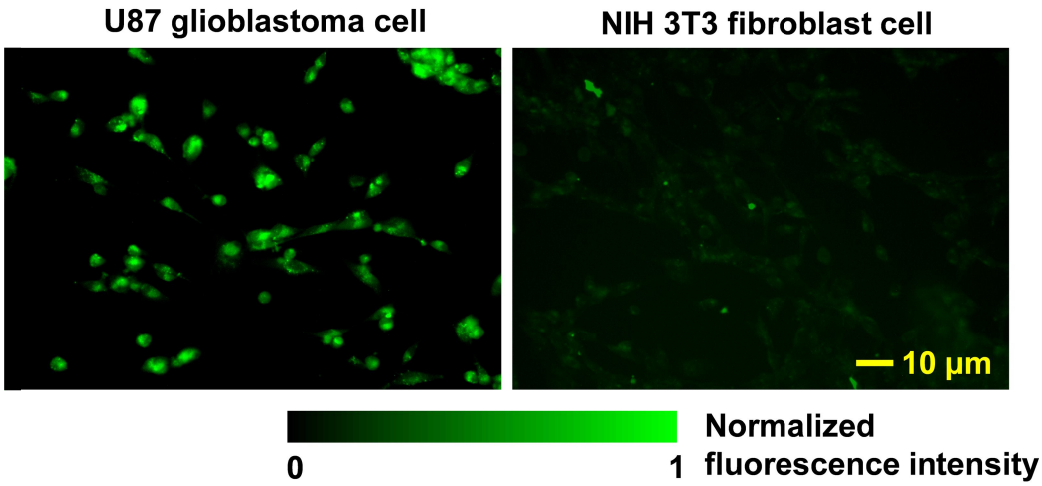

Figure 2. Fluorescence imaging of 2-NBDG uptake by U87 glioblastoma cells and NIH 3T3 fibroblast cells.

Female ND4 Swiss Webster mice were used for the current study. Thirty minutes after the injection of 0.3 mL 3 mM 2-NBDG via the tail vein, stimulations were introduced by two pairs of needle electrodes inserted under the skin of the right and left forepaws, respectively. PACT acquired images continuously at 478 nm and 570 nm. The whole procedure consisted of four periods, each lasting for 3 min (Figure 1c). The first and third periods (P_1 and P_3) were resting states, while the second period (P_2) was right paw stimulation (RPS) and the fourth period (P_4) was left paw stimulation (LPS). Each stimulation period consisted of a train of electrical pulses with an amplitude of 2 mA, a pulse width of 0.25 sec and a repetition rate of 2 Hz.

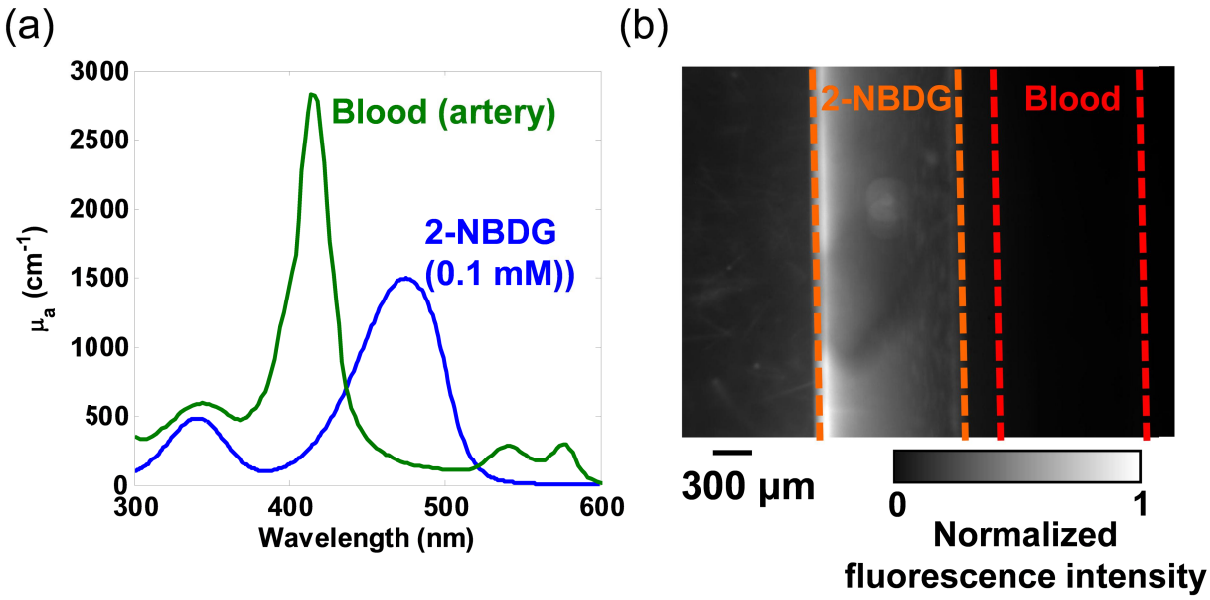

Figure 3. Absorption spectra (a) and fluorescence imaging of bovine blood and 0.1 mM 2-NBDG.

3. RESULTS

At 478 nm, the PA signal amplitudes reflected the 2-NBDG concentration in the brain tissue. At 570 nm, the PA signal amplitude reflected the total hemoglobin concentration in the blood vessels. Capillary-level OR-PAM images of the same mouse are shown in Figure 4. The co-registration results show that the transdermal and transcranial PACT image agreed well with the open-scalp OR-PAM image.

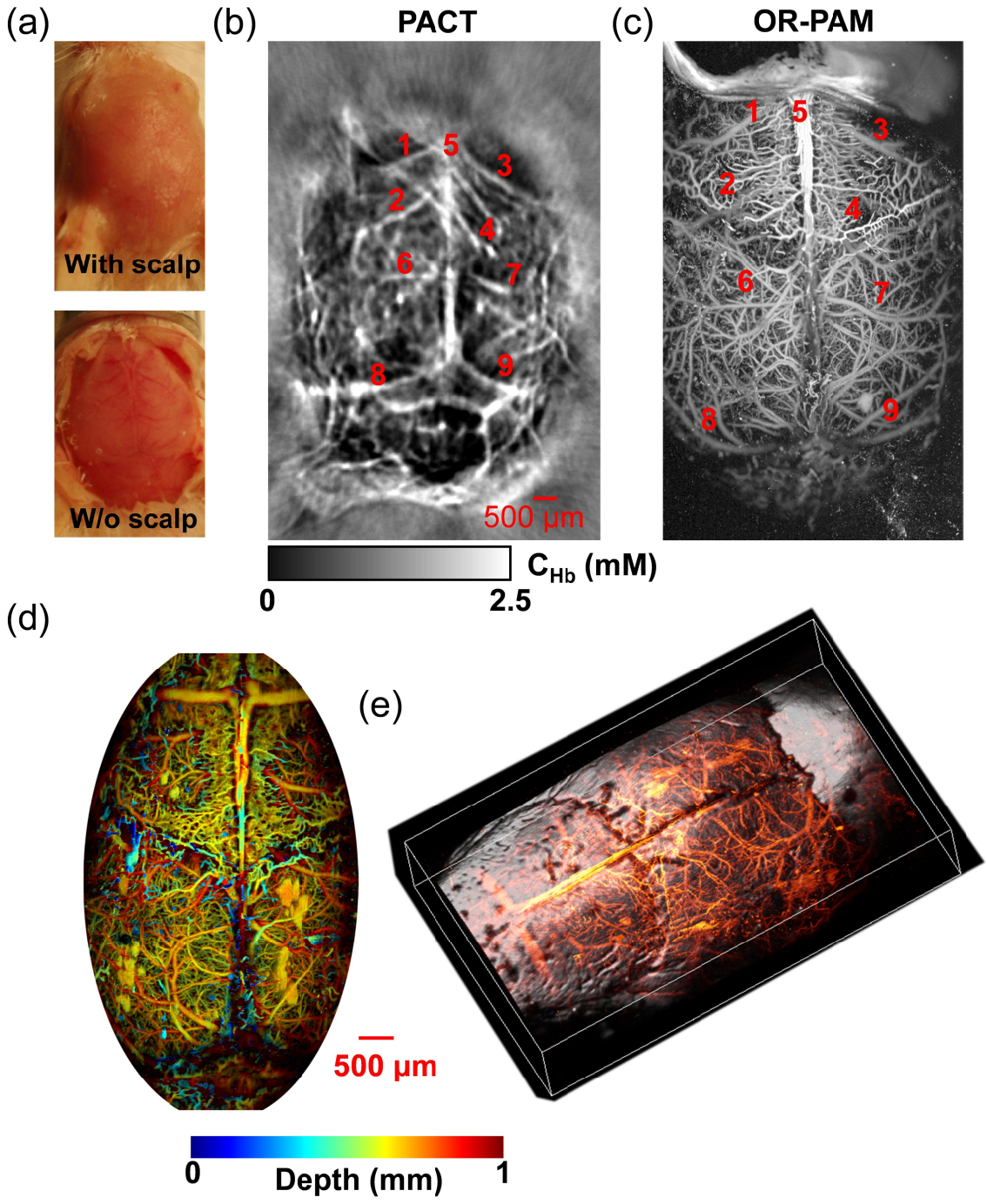

Figure 4. Non-invasive PACT imaging of mouse brain, confirmed by the high resolution OR-PAM imaging with the scalp removed. The numbers in (b) and (c) correspond to the same vascular markers. A depth encoded OR-PAM image is shown in (d) and a volumetric rendering of the photoacoustic and ultrasound images is shown in (e).

Figure 5 shows the relative changes of PA signals induced by forepaw stimulations. At 478 nm, the right paw stimulation (RPS) and left paw stimulation (LPS) caused PA signal amplitudes to increase by 3.6% ± 2.2% and 2.0% ± 1.1% in the somatosensory region (SR) of the contralateral hemisphere, respectively. Such increases indicated elevated

glucose uptake rates, and thus reflected increased neuron activity. Similarly, at 570 nm, the RPS and LPS caused PA signal amplitudes to increase by 6.4% ± 5.3% and 3.2% ± 2.9% in the contralateral hemisphere, respectively. Such changes indicated an increase in total hemoglobin concentration arising from elevated inflows of fresh blood, and thus reflected increased neuron activity as well. In addition, the linear regression shows that the 2-NBDG response amplitude was approximately proportional to that of the hemoglobin response for both RPS and LPS. The high correlation of the two responses may reflect the close coupling between oxygen metabolism and glucose metabolism in the brain.

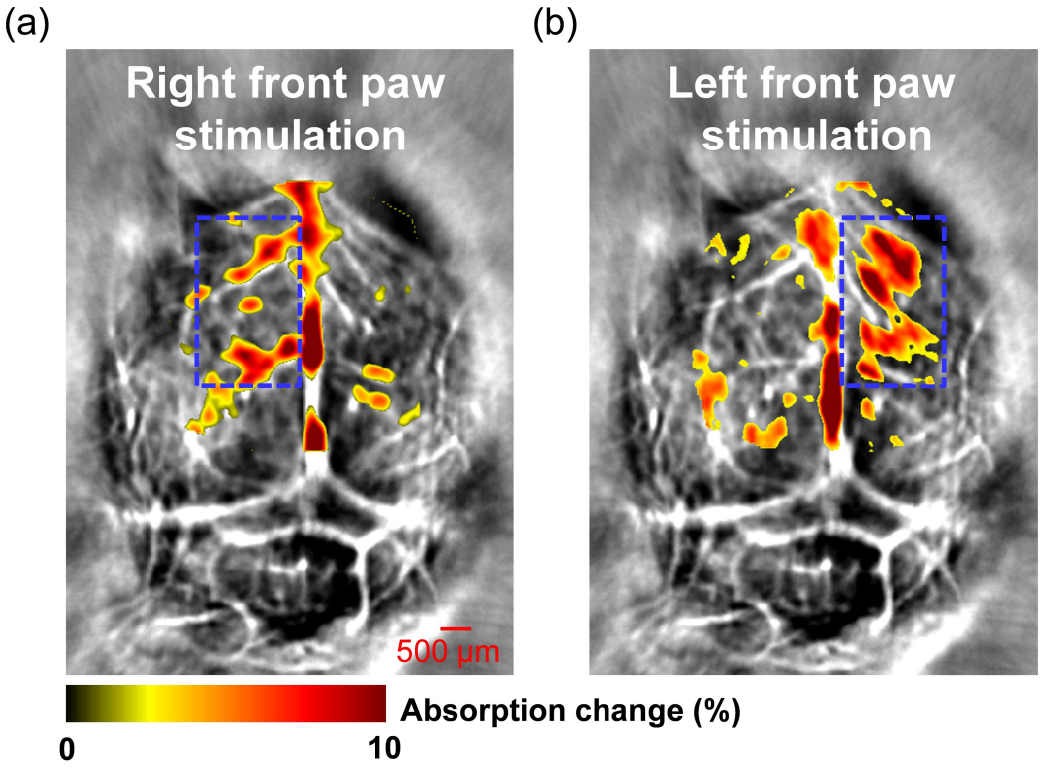

Figure 5. PACT of mouse brain glucose response to electrical stimulations on the forepaws.

4. CONCLUSIONS

In summary, using 2-NBDG as the exogenous contrast and hemoglobin as the endogenous contrast, we have demonstrated that PACT is capable of imaging the metabolic response of a mouse brain to forepaw stimulations. As a quantitative imaging modality, PACT can spectrally separate 2-NBDG and hemoglobin, by virtue of its optical absorption contrast. As a fast imaging modality, PACT can acquire a volumetric image in less than two seconds with a laser repetition rate of 10 Hz. As a deep imaging modality, PACT can transdermally and transcranially localize the spatial patterns of the brain responses by virtue of its high ultrasonic resolution. With all these merits, we expect PACT to be applied to more brain metabolism studies in the future.

5. ACKNOWLEDGEMENTS

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